



Insight on the photocatalytic bacterial inactivation by co-sputtered TiO₂–Cu in aerobic and anaerobic conditions



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ABSTRACT

Co-sputtered TiO₂–Cu polyester (TiO₂–Cu–PES) under actinic light induced bacterial reduction of *Escherichia coli* in the presence of O₂ (air) and under anaerobic conditions. The bacterial inactivation/oxidation proceeds in the absence of O₂ (air) probably due to the highly oxidative TiO₂vb(h⁺) species and the toxic Cu present. By the choice of suitable scavengers, the presence of highly oxidative radicals was confirmed in aerobic media. The *E. coli* inactivation in aerobic media proceeds on TiO₂–Cu–PES within ~30 min and with a slower kinetics of ~90 min in anaerobic media. Malondialdehyde generation a product of bacterial inactivation, was observed on the TiO₂–Cu–PES in air and in lesser amounts under anaerobic conditions. Repetitive bacterial inactivation cycles show a Cu-release of ~2 ppb/cm² by the TiO₂–Cu–PES surface as determined by inductively coupled plasma mass spectrometry (IPC-MS). The Cu released is far below the values reported for the Cu released by TiO₂–Cu–PES samples by sputtering Ti and Cu in sequential order from two targets. By X-ray photoelectron spectroscopy (XPS), redox catalysis by the Cu and TiO₂-species was observed under anaerobic conditions providing further evidence for processes leading to bacterial inactivation in anaerobic media. A mechanism for the TiO₂–Cu–PES bacterial inactivation is suggested consistent with the results reported in this study.

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1. Introduction

The use of TiO₂–Cu nanoparticulate and films in catalysis, photocatalysis and biomedical applications in the last decade is increasing and becoming a topic of interest to many laboratories in the last decade due to their stability, fast kinetics and long term performance. Photocatalysis using TiO₂ combined with Cu have shown to have a stronger performance compared to TiO₂ when eliminating toxic pollutants and when inducing bacterial due to the electron acceptor properties of the Cu-ions. Antibacterial studies reporting some the activity by TiO₂/Cu photocatalysts have recently been reported by Hashimoto/Fujishima [1–4], suggesting that UV-light induces highly oxygenated radicals on TiO₂ damaging the outer cell wall followed by Cu-ion(s) infiltration across the cell membrane in agreement with observations reported by Li and Dennehy [5]

and Ditta et al. [6]. More recently the TiO₂/Cu has been revisited to improve bacterial reduction under light and in the dark addressing the synthesis of uniform, robust adhesive films sputtered on textiles and polymers by our laboratory [7–10]. Several reviews by Foster et al. [11], Page et al. [12], Fujishima et al. [13] and Pelaez et al. [14] have also described the relevance of the TiO₂/Cu semiconductor-metal surfaces in the reduction of variety of pathogens and in conventional catalytic reactions like the decomposition of acetaldehyde [15]. Borkow and Gabbay have recently reported the effective Cu-bactericide textiles modified by Cu-nanoparticulate on different substrates [16]. Our group reported recently on the decoration of binary metal oxides surfaces by Cu enhancing the antibacterial inactivation kinetics [17].

Recently Hashimoto et al. substrates [3,4,13] have reported the preparation of the Cu and TiO₂/Cu films by sol–gel methods inducing significant bacterial inactivation under UV–visible light. Nevertheless, the sol–gel preparations deposited films are not always mechanically stable, not generally reproducible, showing irregular distribution and little adhesion [18–19]. They have been cases where they have been wiped off by a cloth or thumb. On the other hand, recent works by Fisher et al. reported the preparation

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Table 1

Values found by X-ray fluorescence (XRF) for the TiO₂ and Cu content on PES as a function of sputtering time. Reproduced partially from: Applied Catalysis A: General 498 (2015) 185–191 under the license number: 3677140128551.

	wt% Cu/wt PES	wt% CuO/wt PES	wt% Ti/wt PES	wt% TiO ₂ /wt PES
TiO ₂ -Cu (1 min)	0.02	0.04	0.02	0.03
TiO ₂ -Cu (3 min)	0.06	0.07	0.10	0.14
TiO ₂ -Cu (5 min)	0.09	0.11	0.11	0.17

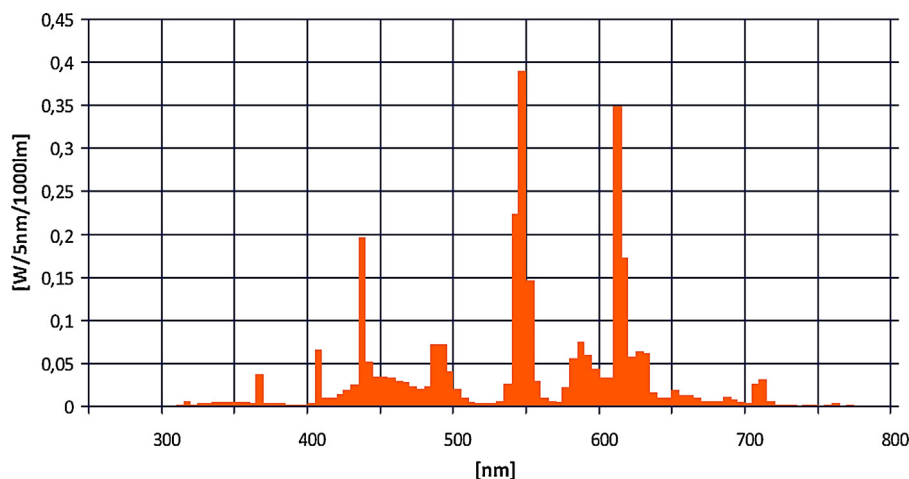


Fig. 1. Emission spectral of the Osram Lumilux 840 actinic lamp source with an irradiation power of 5 mW/cm².

of a robust, immobilised sol-gel Cu/N-TiO₂ coating after annealing at 500 °C [20]. This moved us to work on the preparation of films by magnetron co-sputtering TiO₂ and Cu. Sputtered films have been reported up to now uniform and adhesive surfaces overcoming the shortcomings of colloidal loaded films [21–23]. More important innovative composite are needed to decrease the environmental risks related to healthcare associated infections (HAI) contracted by patients being treated in hospitals. More effective surfaces reducing bacteria in shorter time, avoiding the formation of biofilms in public places should be developed.

Previous work in our laboratory [7] on TiO₂/Cu-PES films reported the preparation of TiO₂/Cu-PES films by sequential DCMS sputtering of TiO₂ for 10 min followed by 40 s sputtering of Cu in an O₂ atmosphere. By profilometry a film thickness of 78 nm was found and the Cu-ion amount in the plasma in the DC-chamber was found of 1–5% as mass spectrometry. A separate study [8] reported at a later date the co-sputtering of TiO₂-Cu-PES films by HIPIMS using a single target 60% TiO₂-40% Cu in an O₂ atmosphere for 150 s. A thinner compact film of 38 nm was obtained. The high power HIPIMS plasma was able to generate an amount of 60–70% Cu-ions besides Cu⁰ and Ar-ions. In a more recent study [9] TiO₂ and Cu were sputtered sequentially in the DCMS chamber for 10 min and 40 s, respectively. A TiO₂-Cu-PES film 60 nm thick in TiO₂ and 38 nm thick in Cu was obtained.

The novelty of the present study consists in the preparation/optimization of co-sputtered TiO₂-Cu-PES films focusing on the study of the bacterial inactivation kinetics under aerobic and anaerobic conditions. The TiO₂-Cu-PES films were investigated: (a) to compare the bacterial inactivation under aerobic and anaerobic bacterial inactivation features, and identify the role of TiO₂vbh⁺ under anaerobic conditions, (b) to report the effect of visible light on the bacterial inactivation compared to the effect of the full actinic light (350–730 nm), (c) to report the protective effect of TiO₂ matrix on the amount Cu-ions leached during bacterial inactivation since by ICP-MS the co-sputtered TiO₂-Cu-PES films reduced drastically the amount of Cu leached compared to the case of the sequential

DCMS co-sputtered TiO₂-Cu-PES films [7] (d) to report the recycling of the TiO₂-Cu-PES films during to bacterial inactivation with a stable 30 min kinetics compared to a less stable kinetics due to the leaching of Cu when sputtering from a single TiO₂-Cu target [8], (e) the identify the oxidative radicals leading to *Escherichia coli* inactivation under aerobic/anaerobic conditions and finally (f) a reaction mechanism is suggested for the interfacial charge transfer (ICFT) leading to bacterial inactivation.

This investigation addresses of co-deposition of TiO₂-Cu films on PES presenting a lower Cu-leaching and concomitantly a more stable TiO₂-Cu-PES film recycling. Novel information on the optical properties is presented and the contact angle reduction during bacterial inactivation and the later recovery of the initial hydrophobic state. The effect of the visible light on aerobic bacterial inactivation on TiO₂-Cu-PES was investigated.

2. Experimental

2.1. Materials and TiO₂-Cu co-puttering on PES in reactive atmosphere

The PES used corresponds to the EMPA test cloth sample No 407. It is a polyester Dacron polyethylene terephthalate, type 54 spun, 130 microns thick, plain weave ISO 105-F04 used for color fastness determination.

A CMS-18 Vacuum system (Kurt Lesker Ltd.) direct current magnetron sputtering was used for the deposition of Ti and Cu in reactive O₂ atmosphere [7–10]. The magnetron chamber was evacuated by way of a turbo-molecular pump. Both the Cu (99.99%) and the Ti (99.99%) targets used were 2 inches in diameter (K. Lesker Ltd., UK). The distance between the targets (Ti and Cu) and the PES substrate was fixed at 10 cm. Co-sputtering was carried out at 300 mA. The nominal calibration of the film thickness was carried out on the Si-wafers and the film thickness was determined with a profilometer (Alphastep 500, TENCOR). Co-sputtering for 3 min

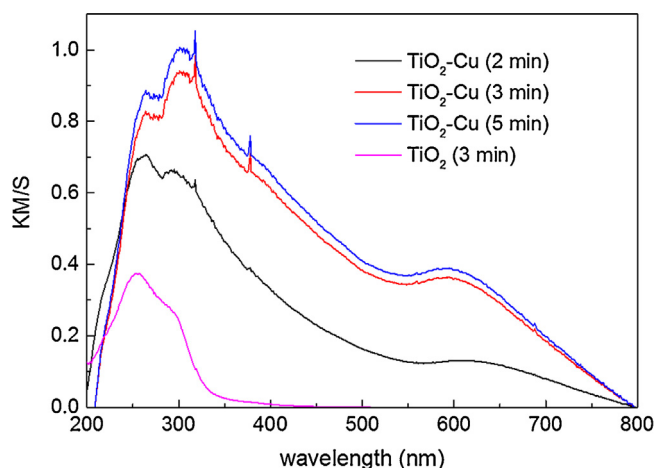


Fig. 2. Diffuse reflectance spectroscopy (DRS) of TiO_2 -Cu co-sputtered and TiO_2 sputtered on PES

of Ti and Cu lead to a coating thickness of ~ 135 nm equivalent to ~ 700 atomic layers [24].

2.2. Determination of the surface composition, diffuse reflectance spectroscopy (DRS) and Cu and Ti released during bacterial inactivation (ICP-MS)

The surface composition was determined by e X-ray fluorescence (XRF) The Ti and Cu on PES was evaluated in a PANalytical PW2400 spectrometer. The results obtained are shown in Table 1.

Diffuse reflectance spectroscopy (DRS) was carried out in a Perkin-Elmer Lambda 900 UV-vis-NIR spectrometer within the wavelength range of 200–800 nm. The rough UV-vis reflectance data cannot be used directly to assess the absorption of the TiO_2 -(Cu/CuO)-PES samples because of the large scattering contribution of the PES fabric to the DRS spectra. Normally a weak dependence is assumed for the scattering coefficient S on the wavelength. The spectra in Fig. 2 were plotted in Kubelka-Munk (KM) units.

A FinniganTM ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) was used to determine the Ti and Cu release during the bactericidal cycles with a resolution of 1.2×10^5 cps/ppb and detection limit of 0.2 ng/L. A washing solution of the TiO_2 -Cu sample were digested with nitric acid 69% (1:1 $\text{HNO}_3 + \text{H}_2\text{O}$) to remove the organics in the solution and to guarantee that there were no remaining ions adhered to the flacon wall. The samples droplets are introduced to the ICP-MS trough a peristaltic pump to the nebulizer chamber at $\sim 7700^\circ\text{C}$ allowing the evaporation and ionization of the elements in the sample. The Cu and Ti found in the nebulizer droplets were subsequently quantified by mass spectrometry.

2.3. Evaluation of bacteria and malondialdehyde during bacterial inactivation and irradiation sources.

E. coli K12 inoculum was prepared by picking bacteria from the colony on the agar plate and incubating them in tryptic broth at 37°C overnight. Overnight cultures of the microorganisms were washed two times in 0.9% NaCl and lately diluted to the selected concentration. *E. coli* K12 strain was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) ATCC23716, Braunschweig, Germany, to test the antibacterial activity of the co-sputtered samples. The PES fabrics were sterilized by autoclaving at 121°C for 2 h. The 20 μL culture aliquots with an initial concentration of $\sim 4.1 \times 10^6$ CFU mL^{-1} in NaCl/KCl (pH 7) were placed on coated and uncoated (control) PES fabric. Samples were then placed on Petri dishes provided with a lid to prevent evapo-

ration. After each determination, the fabric was transferred into a sterile 2 mL Eppendorf tube containing 1 mL autoclaved NaCl/KCl saline solution. This solution was subsequently mixed thoroughly using a Vortex for 3 min. Serial dilutions were made in NaCl/KCl solution. A 100- μL aliquot was pipetted onto a nutrient agar plate and then spread over the surface of the plate using standard plate method. Agar plates were incubated lid down, at 37°C for 24 h before colonies were counted. Three independent assays were done for each sputtered sample and reported in the figures of this study. To verify that no re-growth of *E. coli* occurs after the first bacterial inactivation cycle, the TiO_2 -Cu-film was incubated for 24 h at 37°C . Then, the bacterial suspension of 100 μL was deposited on three Petri dishes to obtain replicates. The samples were incubated at 37°C for 24 h. No bacterial re-growth was observed for these samples.

The bacterial lipid peroxidation leads to the formation of MDA on the TiO_2 -Cu-PES surface. By high performance liquid chromatography (HPLC) the MDA was measured in an Agilent 1100 series HPLC equipped with a UV absorbance detector. Filtered samples were injected via auto-sample and eluted at a flow rate of 0.9 mL/min through a Nucleosil C18 (Macherey-Nagel) column. The mobile phase consisted of a solution 3 mM KH_2PO_4 -MeOH (65–35 v/v%). The MDA peaks in the chromatograms were monitored at 268 nm and the retention time of MDA was 3.9 min.

The irradiation of the *E. coli* bacteria on the co-sputtered samples was carried out by Osram Lumilux 840 actinic lamps as used in hospital facilities with an emission between 340 and 720 nm as shown in Fig. 1 with a light doses of $5 \text{ mW}/\text{cm}^2$.

2.4. The X-ray photoelectron spectroscopy (XPS), change of pH during bacterial reduction measurements and contact angle measurements.

The X-ray photoelectron spectroscopy (XPS) of the TiO_2 -Cu-PES surfaces was determined using an AXIS NOVA photoelectron spectrometer (Kratos Analytical, Manchester, UK) provided for with monochromatic AlK_α ($h\nu = 1486.6 \text{ eV}$) anode. The carbon C1s line with position at 284.6 eV was used as a reference to correct the charging effect. The surface atomic concentration was determined from peak areas using the known sensitivity factors for each element [25]. Spectrum background was corrected according to Nogier et al. [26]. The XPS spectral peaks of TiO_2 were deconvoluted with a CasaXPS-Vision 2, Kratos Analytical UK.

The micro-oxidation analysis followed the change of pH in the junction TiO_2 -Cu-PES followed by the mean of a Jenco 6230N (pH/mV/Temp meter) provided for with a microprocessor and a RS-232-C IBM interface.

The hydrophilicity of the TiO_2 -Cu-PES films was determined by the water droplet contact angle by the sessile drop method on a DataPhysics OCA 35 unit. The measurements were performed from a syringe 1 cm far from the samples (coated and uncoated samples) to reduce the gravity effect of the droplet on the porous PES.

3. Results and discussion

3.1. Diffuse reflection spectroscopy (DRS) and amount TiO_2 -Cu sputtered on PES as a function of time

Table 1 shows the percentage weight if Cu and TiO_2 sputtered at different times on the $2 \times 2 \text{ cm}$ PES samples. When examining the amount of Cu and TiO_2 deposited on PES, TiO_2 denser layers seem to predominate deposited after 3 min compared to the values found after sputtering for 5 min. The increase in the amount of TiO_2 deposited after 3 min sputtering does not increase proportionally to the sputtering time.

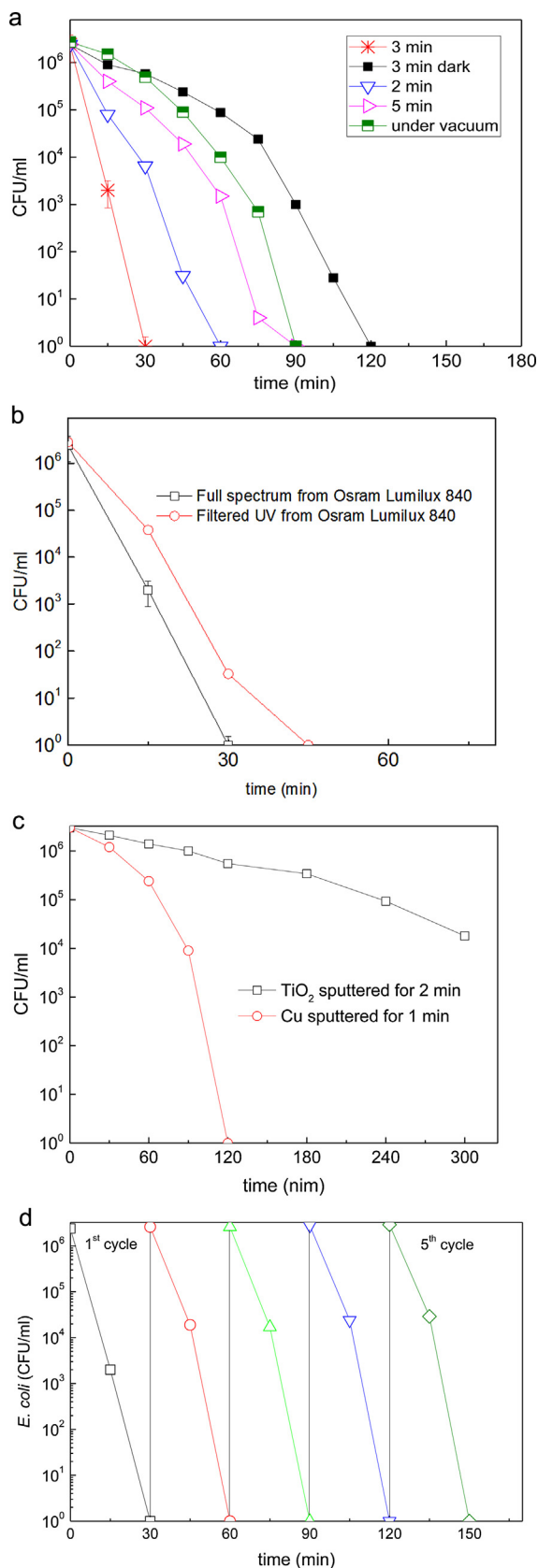


Fig. 3. (a) *E. coli* inactivation on TiO₂-Cu co-sputtered on PES under aerobic conditions for: (—*) 3 min, (—■) 3 min tested in the dark, (—■) 3 min tested under vacuum, (—△) 2 min and (—△) 5 min under Osram Lumilux 840 (5 mW/cm²). Polyester (PES) by itself does not show antibacterial activity. (b) *E. coli* inactivation on co-sputtered TiO₂-Cu-PES (3 min) under light irradiation from: (1) actinic Osram Lumilux 840 (5 mW/cm²) and (2) the same actinic light irradiation but in the presence of a 400 nm cut-off filter. (c) *E. coli* inactivation by TiO₂-PES sputtered

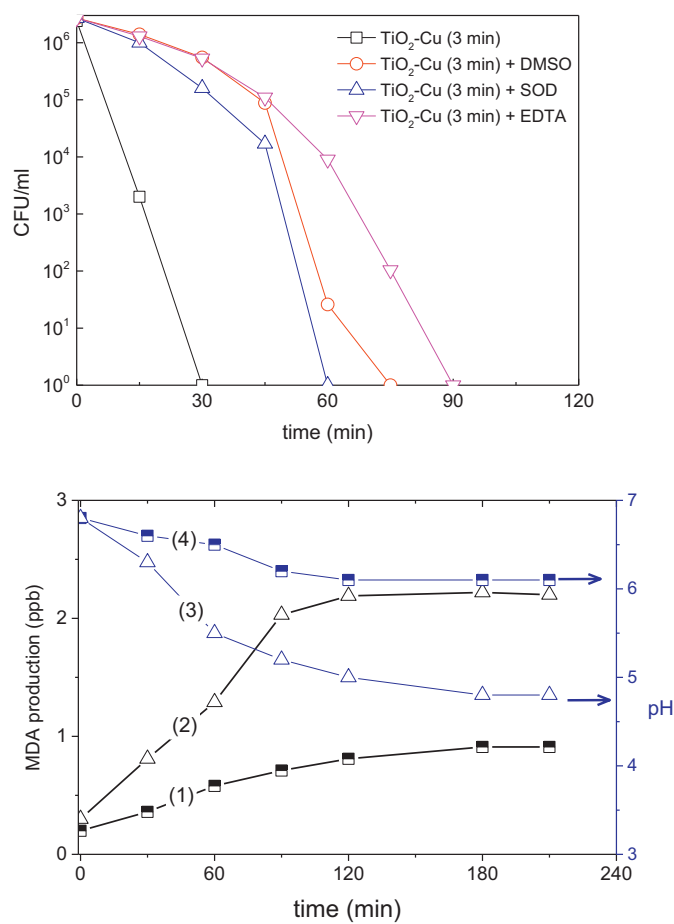


Fig. 4. (a) *E. coli* inactivation in air on TiO₂-Cu-PES co-sputtered for 3 min alone or with radical scavengers: (—■) TiO₂-Cu without scavengers, (—○) TiO₂-Cu with DMSO, (—△) TiO₂-Cu with SOD and (—△) TiO₂-Cu with EDTA. Scavengers were applied on a 0.2 mM concentration. (b) Malonaldehyde (MDA) MDA production under actinic light and local pH changes monitored at the bacteria wall interface during bacterial inactivation on TiO₂-Cu-PES co-sputtered for 3 min: (1) MDA production in anaerobic media (Ar) (2) MDA production in aerobic media (air) (3) local pH change in air and (4) local-pH change under anaerobic conditions (Ar).

The diffuse reflectance spectra (DRS) in Fig. 2 show an increase in the optical absorption as a function of the co-sputtering time in Kubelka-Munk units up to 5 min. The UV-vis reflectance data cannot be used directly to assess the absorption coefficient because of the large scattering contribution to the DRS spectra. Control experiment sputtering only TiO₂ on PES is also shown in Fig. 2.

TiO₂-Cu-PES nanoparticulate have been examined by UV-vis spectroscopy for the first time and shown in Fig. 2. The spectra shown in Fig. 2 are different to the TiO₂ [1] spectrum absorbing only up to 390 nm [1] and the spectra of CuO up to 740 nm [27]. The localized surface plasmon resonance (LSPR) in Fig. 2 are formed when light interacts with the conduction electrons of the TiO₂-Cu leading to collective excitations (oscillations) enhancing the local TiO₂-Cu electromagnetic field. The LSPR broad peak is due to Cu(II)O interband transition at 590 nm (2.1 eV). This peak is due to Cu(I) transitions while the Cu(II) d-d transitions are associated with the exciton bands between 600 and 740 nm [28]. The spectra of TiO₂-Cu blue shifts with longer co-sputtering times as shown in Fig. 2. The interaction of the excited TiO₂-Cu energetic

for 2 min and on Cu-PES sputtered for 1 min in aerobic conditions under Osram Lumilux 840 (5 mW/cm²) light irradiation. (d) Kinetics of the *E. coli* repetitive inactivation on TiO₂-Cu-PES under aerobic conditions irradiated by Osram Lumilux 840 light irradiation (5 mW/cm²).

electron plasmons is a consequence of their distribution on the PES. This plays a role in the interfacial charge transfer between TiO₂ and Cu under actinic light and will be discussed later in Section 3.3 below addressing the photocatalytic mechanism. However, no distinguishable peak was present in the three spectra shown in Fig. 2 due to the absence of Cu-metallic states [29]. Further evidence for this observation will be presented below in Fig. 7 by XPS-measurements.

3.2. Inactivation runs of *E. coli* on TiO₂-Cu-PES under aerobic conditions

Fig. 3a presents the bacterial inactivation kinetics under actinic light on TiO₂-Cu-PES surfaces under aerobic conditions. Complete bacterial reduction is shown in Fig. 3a, within 30 min by TiO₂-Cu co-sputtered on PES for 3 min. Co-sputtering for 2 min did not deposit enough TiO₂-Cu slowing down the bacterial inactivation and co-sputtering TiO₂-Cu for 5 min increased the coating thickness leading to bulk inward charge diffusion induced [13,20]. This explains in both cases the slower bacterial kinetics. The Cu promoter level in the photocatalyst is very low ~0.07 wt% (Table 1) TiO₂-Cu co-sputtered for 3 min. But 5 min co-sputtering increases the amount of Cu > 50% and seems to act as a charge recombination center. Co-sputtering for 3 min would allow the Cu present to suppress the electron/hole recombination in TiO₂ acting as a trap for the generated charges and enhancing the charge separation. The *E. coli* reduction in the dark in Fig. 3a occurs within 120 min due to the lack of TiO₂ contribution in the TiO₂-Cu-PES samples [4,8]. Fig. 3a shows that complete bacterial inactivation is attained within 90 min under anaerobic conditions.

Fig. 3b shows the *E. coli* inactivation time by the actinic light used so far and also by the same light source when introducing 400 nm cut-off filter between the light source and the sample. The results obtained show that a 50% longer kinetics is required to inactivate bacteria in the presence of the UV cut-off filter.

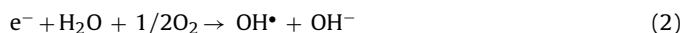
Fig. 3c TiO₂-PES shows the inactivation time by TiO₂-Cu-PES sputtered for 2 min and for Cu-PES sputtered for 1 min under aerobic conditions under Osram Lumilux 840 (5 mW/cm²) light irradiation. The TiO₂ sputtered for 2 min and the Cu sputtered for 1 min was chosen to correspond to the amount of TiO₂ and Cu present in TiO₂-Cu samples co-sputtered for 3 min. Simultaneous deposition of TiO₂-Cu on PES for 3 min led to the best bacterial inactivation kinetics (30 min) as shown in Fig. 3a. By XRF the catalyst shows a composition of 2/3 TiO₂ and 1/3Cu. So to show that a synergistic effect led to the 30 min bacterial inactivation, we applied both TiO₂ (for 2 min) and Cu (for 1 min) separately on PES. The results are shown in Fig. 3c leading to longer bacterial inactivation times compared to the 3 min co-sputtered TiO₂-Cu. The results in Fig. 3c suggest that actinic light induces highly oxygenated radicals on TiO₂ leading to *E. coli* damage. This damage allows Cu-ion(s) translocation/permeation across the cell membrane in agreement with work reported by Hashimoto/Fujishima et al. [1–4], Li et al. [5] and Ditta et al. [6].

The stability of the TiO₂-Cu-PES performance during repetitive *E. coli* inactivation is shown in Fig. 3d. The bacterial inactivation is seen to proceed with the same kinetics. The results presented in Fig. 3d suggest then use of the TiO₂-Cu samples against nosocomial infections. The level of contamination in public hospitals in the UK has been reported to higher than the allowed level in the hospital rooms [11,13,20]. The contamination of 10⁵ CFU/cm² was observed in diabetic wound dressing rooms. But in the vicinity of the patient, a microbial density of 10² CFU/cm² was found and therefore nosocomial infections should be effectively reduced by the TiO₂-Cu-PES films presented in this study Table 2.

3.3. Scavenging of the oxidative radicals under aerobic and anaerobic conditions, photoproduct formation, local pH changes, reversible photo-switching during bacterial inactivation

Photocatalytic inactivation of bacteria in aerobic conditions proceeds by highly oxidative radicals OH•, HO₂•/O₂^{•-} and TiO₂cb(h⁺). Fig. 4a present the scavenging experiments by dimethylsulfoxide (DMSO), superoxide dismutase (SOD) and Ethylene tetra-acetic acid di-sodium salt (EDTA-2Na) to detect the role of the OH•, O₂^{•-} and TiO₂ cb(h⁺) respectively in bacterial oxidation. It is seen that the bacterial inactivation mediated by TiO₂-Cu-PES alone proceeds within 30 min, but the kinetics slows down in the presence of the 3 added scavengers. This means that without any added scavengers the bacterial inactivation proceeded via precursors originating from Cu/Cu-ions and TiO₂. Adding DMSO and SOD to the bacteria on TiO₂-Cu-PES did not change the 30 min bacterial inactivation time as reported for pure TiO₂-Cu-PES in Fig. 4a. Therefore, the toxicity effect of the Cu/Cu-ions and the TiO₂cb(h⁺) led to bacterial inactivation in anaerobic conditions.

Fig. 4b shows the evolution in aerobic and anaerobic media of the malondialdehyde generation a known decomposition product of bacterial [30]. The measure of MDA is the absorbance of the absorbance of the strong colored band after the treatment of MDA with TBA [8,30]. The TBA forms with MDA a pink complex at 532 nm with $\epsilon = 49,500 \text{ M}^{-1} \text{ cm}^{-1}$ linearly proportional to lipid peroxidation of *E. coli*. The most important observation reported in Fig. 4a is that MDA can be formed under anaerobic conditions on the TiO₂-Cu-PES. Fig. 4b shows the change of pH in the outer cell wall pH up to 200 min occurring during bacterial inactivation towards more acidic values. In Fig. 4, It is readily seen that in aerobic media, the pH decreases stepwise from ~6.9 to ~4.8. This is equivalent to an increase of two orders of magnitude in the proton concentration during bacterial inactivation. In anaerobic conditions the pH decreases from a pH 6.8 to 6.1, less than one order of magnitude. Fig. 4b shows that of H⁺ generation predominates over OH⁻ since a displacement to more acidic pH-values was observed during bacterial inactivation. The TiO₂ vb(h⁺) oxidize the surface Ti-OH to OH• and since the water chemisorbed on the TiO₂ leads to the hole transfer. Concomitantly reactions (2–4) show follow up reactions leading to the generation of OH• and O₂^{•-} species. Reaction (3) at pH > 5 leads to O₂^{•-} due to the equilibrium HO₂• → O₂^{•-} + H⁺ (pK_a = 4.8). Reaction (4) does not lead to H₂O₂ since Fenton-like reactions set in due to the Cu-released during bacterial inactivation as shown in Fig. 5 below. The H₂O₂ decomposition have been reported in the presence of Cu(I)O/Cu(II)O [31].



The interfacial charge transfer from TiO₂ to Cu/CuO induced by light has been reported before [4,8]. It will not be discussed in a detailed way in this study. IFCT has been reported to take place with high quantum efficiency. A short notation of the reactions taking place can be stated as: Under actinic light (see Fig. 1), the CuO can be reduced to Cu₂O and the Cu₂O reduces O₂ via a multi-electron process and re-oxidizes itself to CuO. The charges generated by light in the TiO₂-Cu reduce O₂ and CuO at the CuOcb. The conduction band of CuO at -0.30 V vs SCE (pH 7) is at a more negative potential than the potential required for the one electron oxygen reduction O₂ + H⁺ + e⁻ → HO₂• - 0.22 V [4,13]. Furthermore, the Cu²⁺ can react with e⁻ (or O₂^{•-}) → Cu⁺ + (or O₂). The Cu⁺ can reduce O₂ consuming electrons or be oxidized to Cu-ions by the photo-generated TiO₂ holes to Cu²⁺ [20]. The TiO₂vb holes react with the surface-OH of

Table 2
Surface atomic % on TiO₂-Cu-PES co-sputtered for 3 min before and after bacterial inactivation in aerobic and anaerobic conditions. Reproduced partially from: Applied Catalysis A: General 498 (2015) 185–191 under the license number: 3677140128551.

		C	O	N	Cu	Ti	Ar
Aerobic conditions	Before bacterial inactivation	31.8	22.1	0.7	19.3	26.1	0
	After bacterial inactivation	39	19.4	0.9	16.6	24.1	0
Anaerobic conditions	Before bacterial inactivation	35.3	7.5	0.7	19.9	27.9	8.5
	After bacterial inactivation	46.5	9.1	0.8	16.6	24.7	2.3

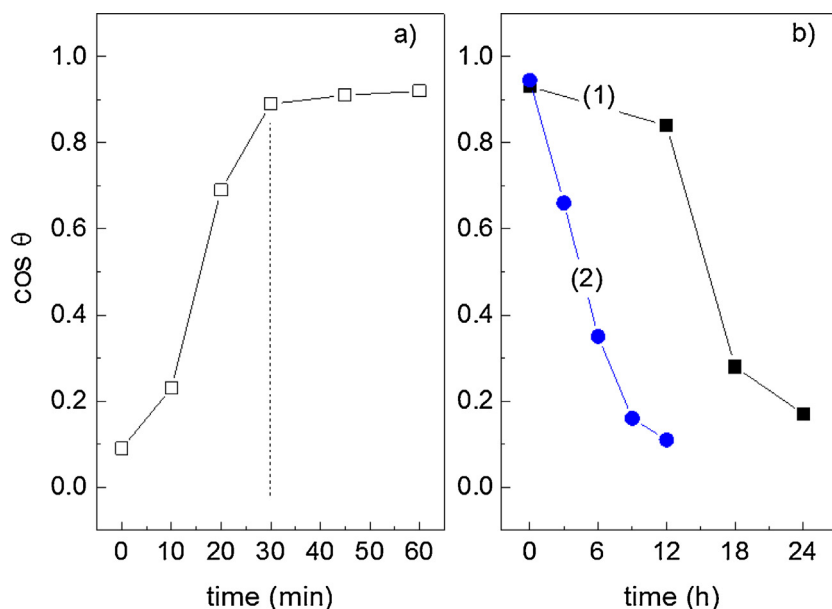


Fig. 5. (a) Contact angle variation due to the hydrophobic-hydrophilic transformation on the on co-sputtered TiO₂-Cu-PES (3 min) under Osram Lumilux 840 (5 mW/cm²) during bacterial inactivation and (b) dark reverse hydrophilic-hydrophobic back reaction kinetics after bacterial inactivation samples towards the initial hydrophobic state: (1) at room temperature, 24 °C and (2) at 60 °C.

the TiO₂ releasing OH-radicals to inactivate bacteria as shown in Reaction (1) [8,20].

Fig. 5a shows the hydrophobic to hydrophilic transformation as a function of $\cos\theta$ by the TiO₂-Cu-PES film due to actinic light irradiation within 60 min. For any practical purposes, the initial hydrophobic surface is converted to a super-hydrophilic surface within 30 min. Fig. 5b shows that the back transformation to the hydrophobic state in the dark as a function of $\cos\theta$. The reverse reaction was almost complete within 24 h. These rates were calculated by integrating “ $\cos\theta$ ” in the Young’s equation [13,20].

According to Young’s theory the “ $\cos\theta$ ” of a liquid droplet on a solid is a function of the interfacial energy between the solid and the liquid. The wettability is commonly evaluated in terms of the contact angle (CA) which is given by Young’s equation [8]:

$$\gamma S = \gamma SL + \gamma L \times \cos\theta \quad (5)$$

In Eq. (5) γS and γL are the surface free energies per unit area of the solid and liquid respectively, and γSL is the interfacial free energy per unit area. In addition, γSL can be approximated using the Girifalco–Good equation, with γS and γL , as:

$$\gamma SL = S + L - \Phi(\gamma S \gamma L)^{1/2} \quad (6)$$

Here, Φ is a constant parameter ranging from 0.6 to 1.1, depending on the solid. In addition, γL is the water surface free energy, which has a constant value of 74 mJ/m². Therefore, by combining (5) and (6), the CA can be simply expressed as:

$$\cos\theta = c\gamma S^{1/2} - 1 \quad (c : \text{constant}) \quad (7)$$

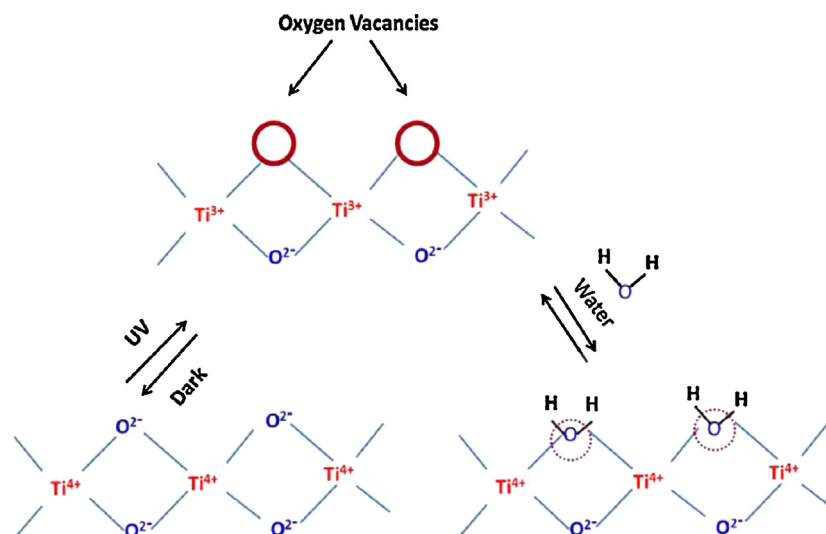
The highly hydrophilic state generated by UV light gradually returns to the initial hydrophobic state in the dark shown in Fig. 5b.

The nature of the film has been reported to play an important role in bacterial adhesion. *E. coli* (Gram (–)) and *Staphylococcus aureus* (Gram (+)) present a preferential adhesion to hydrophilic surfaces [8]. The hydrophobic-hydrophilic transformation is induced by the photo-generated holes of the TiO₂ involve trapped lattice oxygen sites [13,20]. Fig. 5b shows the complete transformation back to hydrophilicity within 24 h. This result suggests that to full performance during the catalyst recycling will proceed only upon restoration of the initial hydrophobic surface within 24 h in the dark. Samples kept in the dark under 60 °C led to faster hydrophobicity restoration on the sputtered film. This can be explained by the faster water evaporation leading to faster restoration of the initial state on the TiO₂-Cu film. In the absence of light irradiation, TiO₂-Cu-PES restoration of the initial hydrophobic within 24 h, due to the gradual desorption of hydroxyl groups from the surface in the form of H₂O₂, H₂O and O₂ as reported recently by Banerjee et al. [21]. Scheme 1 shows the mechanism of the hydrophilic transformation and the dark back transformation to the initial hydrophobic state.

In Scheme 1 (Reproduced from App. Cat B 176–177 (2015) 396–428) under the license No 370700040146) the electrons reduce the Ti⁴⁺ to Ti³⁺ creating oxygen vacancies in the TiO₂ network. These oxygen vacancies increase the affinity for water as the surface becomes more hydrophilic.

3.4. Monitoring the release of Cu and Ti by ICP-MS and the change in oxidation states of Cu and TiO₂ during bacterial inactivation

The release of Cu and Ti from a TiO₂-Cu-PES sample under light during repetitive bacterial inactivation is shown in Fig. 6. The level



Scheme 1. Schematic representation of photo-induced hydrophilicity. Electrons reduce the Ti(IV)–Ti(III) state and thereby the oxygen atoms will be ejected (creation of oxygen vacancies) increasing the affinity for water molecules and thereby transforming the surface hydrophilic.

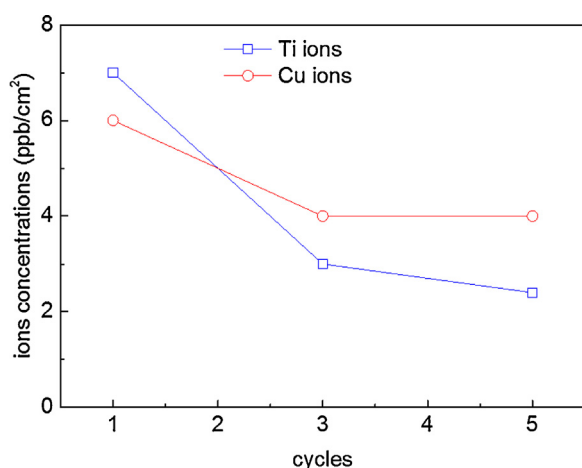


Fig. 6. Inductively plasma coupled mass spectrometry (ICP-MS) determination of the Cu and Ti-ions released during bacterial inactivation from a co-sputtered TiO₂–Cu–PES (3 min) sample up to the 5th recycling.

of Cu-release drops from 6 to about 4 ppb/cm² after the 5th cycle. For Ti-released concomitantly also drops from 7 to 2 ppb/cm² after the 5th cycle.

The Cu and Ti-leached out during *E. coli* bacterial reduction occur at very low levels below the cytotoxic levels for Cu possibly involving a quasi-oligodynamic effect. The low release Cu of 4 ppb/cm² is far below 25 ppb/cm² determined for the threshold of Cu-cytocompatibility for mammalian cells [32]. Cu levels of 4 ppb/cm² introduce an oligodynamic effect when reducing *E. coli* and MRSA [33]. The Cu-ions have been reported to bind S, N and COO[−] and other electron donor negative groups of the bacteria cell wall or entering the bacteria cytoplasm. Due to their small size the Cu-ions are able go through the 1.0–1.1 nm porins allowing the Cu-ion translocation across the cell wall membrane into the bacterial cytoplasm [34]. Other bacterial inactivation channels during the direct contact between the Cu- and the bacteria outer cell wall also contribute to bacterial reduction [35].

Fig. 7 presents the deconvoluted Cu2p doublet at time zero and after bacterial inactivation in anaerobic media. This doublet is attributed to the presence of the Cu²⁺-oxidation state (Cu²⁺) prevalent in the samples exposed to air [7–10]. The CuO with BE 932.1 eV at time zero remains stable after bacteria reduction

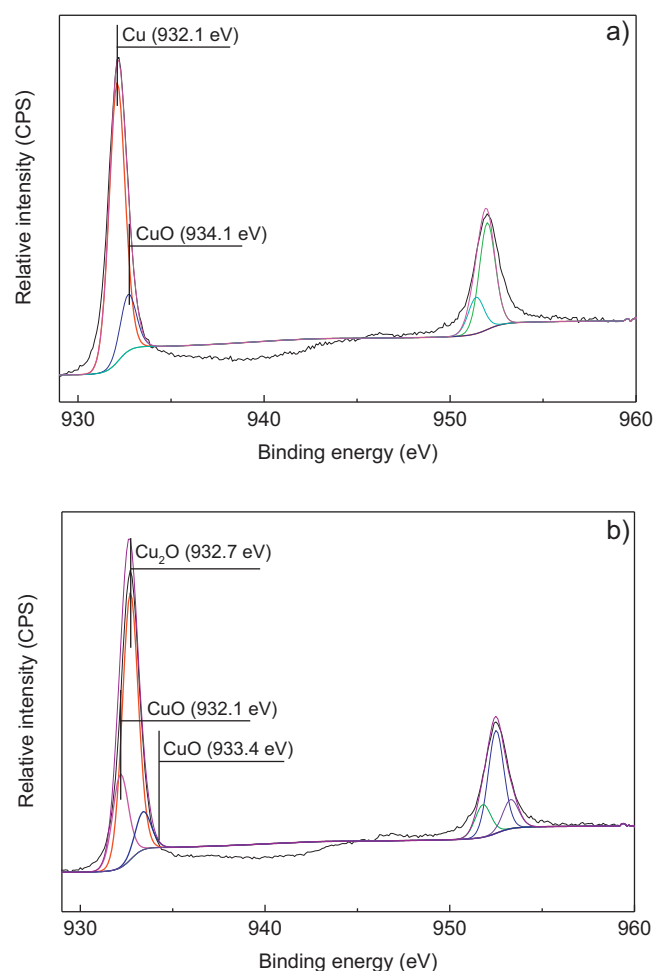


Fig. 7. XPS deconvolution of Cu2p envelope in TiO₂–Cu–PES in anaerobic conditions before and after 30 min bacterial inactivation for a sample irradiated by Osram Lumilux 840 light irradiation (5 mW/cm²).

since the CuO BE is conserved. A new peak ascribed to Cu₂O after 30 min bacterial reduction at 932.3 eV in Fig. 6b. An XPS shift of >0.2 eV is indicative of a change in the oxidation state in the XPS

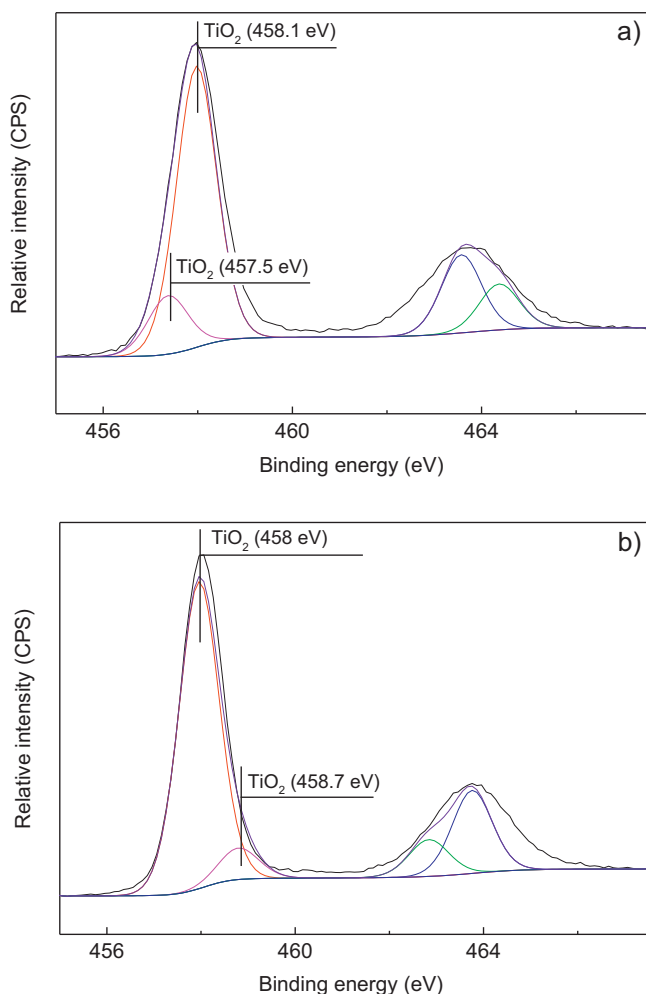


Fig. 8. XPS deconvolution of Ti2p envelope in TiO₂-Cu-PES in anaerobic conditions before and after 30 min bacterial inactivation for a sample irradiated by Osram Lumilux 840 light irradiation (5 mW/cm²).

specie [25,26]. Redox catalysis takes place therefore during the disinfection process in spite of the O₂ (air) exclusion. This observation lends further support to the data reported in Figs. 3a and 4 showing that redox reactions leading to bacterial reduction can be induced by actinic light in anaerobic media. This is to our knowledge the first evidence for this type of reaction occurring in anaerobic media. Recently Cu₂O has been reported as being effective antibacterial/anti-fungi/anti-phage/antiviral agent when prepared by sol-gel methods or used as supported powders on glass [36–37].

Fig. 8 shows that after bacterial reduction a new TiO₂ peak appears with a different BE respect to the peak found at time zero in Fig. 7a. The redox chemistry is associated with the production of highly oxidative radicals by TiO₂-Cu-composites [4,8]. Redox processes in TiO₂ usually involve Ti³⁺/Ti⁴⁺ surface electron traps enhancing O₂ chemisorption [13,20]. Fig. 4a showed high oxidative radicals intervening in the *E. coli* inactivation. The TiO₂ to Cu electron transfer (IFCT) will probably enhance redox interactions due to more energetic electrons intervening in the bacteria inactivation.

Conclusions

This study reports on the heterogeneous *E. coli* oxidation under anaerobic conditions on TiO₂-Cu-PES co-sputtered surface. This bacteria inactivation proceeds with a faster kinetics in aerobic

media compared to anaerobic media. Adding DMSO and SOD in aerobic media under light irradiation in the presence of TiO₂-Cu-PES slowed down the bacteria inactivation time. Under anaerobic conditions the addition of DMSO and SOD did not change the bacterial inactivation period. This means that the TiO₂vb(h⁺) species and the toxicity of the Cu-ion are responsible for *E. coli* inactivation under anaerobic conditions. The hydrophobic-hydrophilic transformation of the TiO₂-Cu-PES surface was seen to be complete within 30 min, the same time needed for bacterial inactivation. Recovery of the full hydrophobic character of the TiO₂-Cu-PES samples required 24 h. The TiO₂-Cu-PES co-sputtered catalyst was shown to recycle and destroy *E. coli* with the same kinetic rate over several cycles. This was not the case for The TiO₂-Cu-PES films sputtered sequentially by TiO₂ followed by Cu.

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